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Quality of AAV Gene Therapy Products from a Regulator´s view

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Disclaimer

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Do we already know every AAV Quality Attribute?

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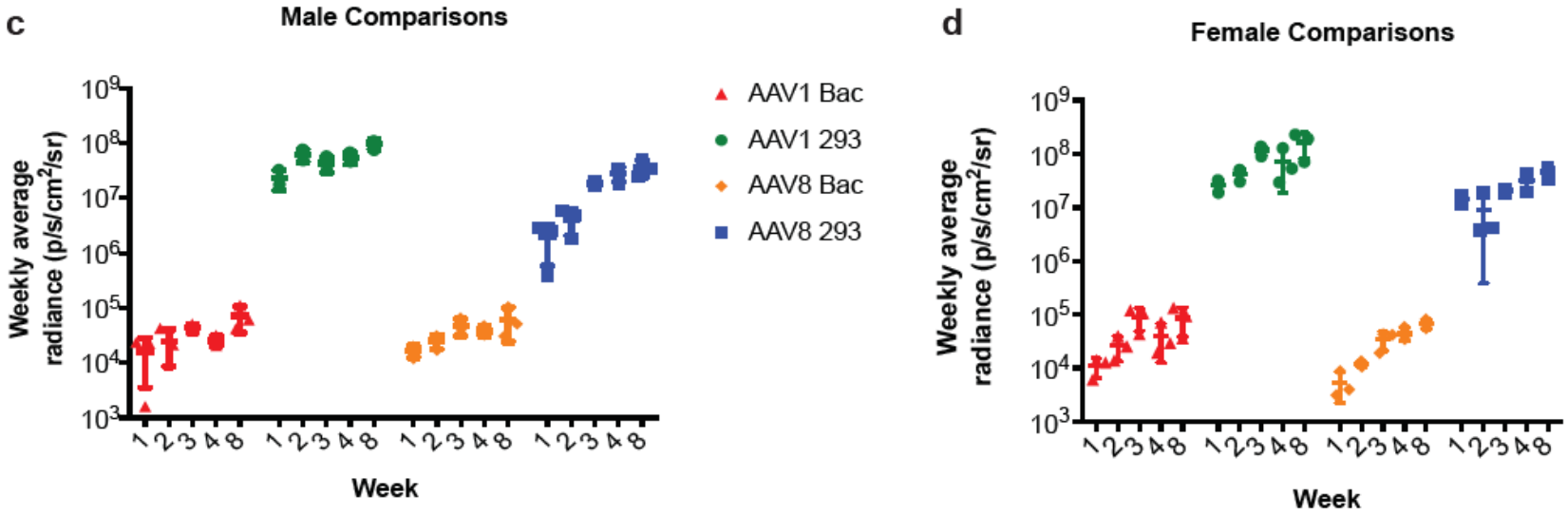
Uncertainties remain



- AAV from human- versus insect cell manufacturing platforms show higher potency *in-vivo* (mice)
- Differences in impurities or post translational modifications only partly explain that phenomenon

⇒ Seems that not all important Quality Attributes of AAV products are known

Source: RUMACHIK, Neil G., et al. Methods Matter--Standard Production Platforms For Recombinant AAV Can Produce Chemically And Functionally Distinct Vectors. bioRxiv, 2019, S. 640169

Characterisation: As good as possible

Set of Quality Attributes to test

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AAV Genome

- AAV genome sequence – Sanger Sequencing - ITR to ITR
- Unwanted packaged sequences – Screening with deep sequencing and confirmation with qPCR
- Ratio of positive to negative DNA strands
- Vector genome size – alkaline electrophoresis – Size variants distribution
- Vector genome titer – qPCR, SEC-HPLC
- Empty/Full capsid – Analytical Ultracentrifugation

More/different trouble when nearing the packaging limit of AAV at about 4.7 kb

Characterisation: As good as possible

Set of Quality Attributes to test

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AAV Capsid

- AAV capsid protein sequence – peptide mapping – LC-MS/MS
- Capsid titer – ELISA, SEC-HPLC
- VP secondary structure – far-UV circular dichroism
- VP thermal stability – far-UV circular dichroism
- Capsid integrity – Release of DNA at increasing temperatures

Post-translational modifications

- Percent capsid deamidation – LC-MS
- Percent capsid oxidation – LC-MS

Characterisation: As good as possible

Set of Quality Attributes to test

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AAV Capsid

Capsid architecture

- Ratio of VP1, VP2 and VP3 – reverse-phase HPLC
- AAV particle size and polydispersity – dynamic light scattering
- AAV morphology – Cryo-TEM

Biological Activity

- Potency Assay – Difficult if gene product is a structural element of a cell/tissue
- Expression Assay – Not always indicative for potency
- TCID50 – Highly variable

Impurities

- Product related
- Process related

Characterisation: Future?

New/refined methods to get a better picture of the AAV product

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Revolutionary cryo-EM is taking over structural biology

The number of protein structures being determined by cryo-electron microscopy is growing at an explosive rate.

A revolutionary technique for determining the 3D shape of proteins is booming. Last week, a database that collects protein and other molecular structures determined by cryo-electron microscopy, or cryo-EM, acquired its 10,000th entry.

<u>Cryo-EM:</u>
No crystallization of samples needed - Application for characterization of AAV possible and useful?

<u>Post-Translational modifications</u>
Which are functional significant and how to best quantify them?

<u>Finding what we do not know…</u>

Conclusion

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<u>Quality Assessment during MAA profits from:</u>

Reducing uncertainty concerning the quality of the product over the manufacturing development

⇒ Covering a wide variety of Quality Attributes when characterising AAV product

⇒ Using current state-of-the-art analytical methods

Thank you for your attention!

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